hundred microliters each of DOPE:DODAC:PEG-Cer(C_{20}) control particles was injected into one group of three female ICR mice and 200 μL of DOPE:DODAC:PEG-Cer(C_{14}) control particles was injected into three groups of three of the ICR mice. The plasma was analyzed for ^{14}C-lipid after 1, 2 and 5 hours.

FIG. **20** shows the clearance of DNA encapsulated in particles composed of DOPE:DODAC:PEG-Cer(C_{20}) ((83.5:6.5:10 mole %). The DNA and lipid are cleared much less rapidly from the circulation than when PEG-Cer(C_{14}) is used (see FIG. **21**). Nearly 50% of the lipid and DNA are present after 1 hour. A significant amount of DNA and lipid were still present after 5 hr. The amount of DNA and lipid injected was 1.8 μg and 853 μg, respectively. Control particles exhibited a clearance similar to that of the plasmid-lipid particles.

FIG. **21** shows the clearance of DNA encapsulated in particles composed of DOPE:DODAC:PEG-Cer(C_{14}) ((83.5:6.5:10 mole %). Both DNA and lipid are 5 cleared rapidly from the circulation with only about 20% of the lipid and 10% of the DNA present in the plasma after 1 hr. The amount of DNA and lipid injected was 2.7 μg and 912 μg, respectively. Control particles exhibited a clearance similar to that of the plasmid-lipid particles.

In Vivo Transfection in Lung, Liver and Spleen

Three groups of four IRC mice were injected via tail vein with pCMV4-CAT encapsulated in lipid particles composed of DOPE:DODAC:PEG-Cer(C_{14}) (83.5:6.5:10 mole %, "A") or DOPE:DODAC:PEG-Cer(C_{20}) (83.5:6.5:10 mole %, "B"), prepared as described above. The mice were sacrificed after 2, 4 and 8 days and the lung, liver and spleen were assayed for CAT activity according to a modification of Deigh, *Anal. Biochem.* 156:251–256 (1986). The amount of plasmid injected was 2.6 μg for the particles containing PEG-Cer(C_{14}) and 1.5 μg for the particles containing PEG-Cer(C_{20}).

FIG. **22** shows the results of in vivo transfection achieved in the lung. As can be seen from this figure, treatment with formulation "A" provided excellent transfection efficiency (based on CAT activity) up to 4 days. Formulation "B", while resulting in overall lower levels of CAT activity, provided relatively constant levels of enzyme activity over 8 days.

FIG. **23** shows the results of transfection achieved in the liver. For both formulations, transfection (and CAT activity) reached a maximum at 4 days.

FIG. **24** shows the results of transfection achieved in the spleen wherein the maximum transfection was found for both formulations to occur after 2 days.

VII. Conclusion

As discussed above, in accordance with one of its aspects, the present invention provides methods for preparing serum-stable plasmid-lipid particles which are useful for the transfection of cells, both in vitro and in vivo.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. A method for the preparation of serum-stable plasmid-lipid particles, comprising:

(a) combining a plasmid with cationic lipids in a detergent solution to provide a coated plasmid-lipid complex;

(b) contacting non-cationic lipids with said coated plasmid-lipid complex to provide a solution comprising detergent, a plasmid-lipid complex and non-cationic lipids; and

(c) removing said detergent from said solution of step (b) to provide a solution of serum-stable plasmid-lipid particles, wherein said plasmid is encapsulated in a lipid bilayer and said particles are resistant to degradation in serum, and wherein the particles have a diameter ranging from about 50 to about 150 nm.

2. A method in accordance with claim **1**, wherein said removing is by dialysis.

3. A method in accordance with claim **1**, wherein step (b) further comprises adding a polyethylene glycol-lipid conjugate.

4. A method in accordance with claim **3**, wherein said polyethylene glycol-lipid conjugate is a PEG-ceramide conjugate.

5. A method in accordance with claim **1**, further comprising;

(d) sizing said particles to achieve a uniform particle size.

6. A method in accordance with claim **1**, wherein said cationic lipids are selected from the group consisting of DODAC, DDAB, DOTAP, DOTMA, DOSPA, DOGS, DC-Chol and combinations thereof.

7. A method in accordance with claim **1**, wherein said non-cationic lipids are selected from the group consisting of DOPE, POPC, EPC and combinations thereof.

8. A method in accordance with claim **1**, wherein said detergent solution comprises a detergent having a critical micelle concentration of between about 20 mM and 50 mM.

9. A method in accordance with claim **8**, wherein said detergent is n-octyl-β-D-glucopyranoside.

10. A method for introducing a plasmid into a cell, comprising:

(a) preparing a plasmid-lipid particle according to the method of claim **1**; and

(b) contacting said cell with said plasmid-lipid particle for a period of time sufficient to introduce said plasmid into said cell.

11. A method in accordance with claim **10**, wherein said plasmid-lipid particle comprises a plasmid, DODAC, POPC and a PEG-Ceramide selected from the group consisting of PEG-Cer-C_{20} and PEG-Cer-C_{14}.

12. A method in accordance with claim **10**, wherein said plasmid-lipid particle comprises a plasmid, DODAC, DOPE and a PEG-Ceramide selected from the group consisting of PEG-Cer-C_{20} and PEG-Cer-C_{14}.

13. In a method of gene therapy involving the introduction of a plasmid via a plasmid-lipid composition into a cell resulting in sufficient expression to effect a phenotypic change, the improvement which comprises

(a) preparing a plasmid-lipid particle according to the method of claim **1**; and

(b) contacting said cell with said plasmid-lipid particle for a period of time sufficient to introduce said plasmid into said cell.

14. A method for the preparation of serum-stable plasmid-lipid particles, comprising:

(a) preparing a mixture comprising cationic lipids and non-cationic lipids in an organic solvent;